ON SOME FACTORS GOVERNING THE PERMEABILITY OF THE EGG MEMBRANE BY THE SPERM.

H. H. NEWMAN.

In the summer of 1903 Loeb[1] succeeded, by slightly increasing the alkalinity of sea water, in fertilizing the eggs of the sea-urchin (*Strongylocentrotus purpuratus*) with the sperm of the 20-rayed starfish, with that of Asterias and with that of the Holothurian, *Cucumaria*, while at the same time the eggs were rendered immune to their own sperm.

The question arose as to the character of the change in the egg or in the sperm that makes the former sometimes permeable to its own sperm and impermeable to foreign sperm and sometimes impermeable to its own sperm but quite freely permeable to the sperm of other species.

In one place Loeb tentatively suggests, "that the whole effect of the alkali is simply to bring about a slight physical change in the condition of the protoplasm of the egg, or the surface of the spermatozoön, which facilitates the entrance of the latter into the egg"; and in a continuation of the same series of experiments,[2] published some months later, he suggests "that the main variable determining the entrance of the spermatozoön into the egg may be of the nature of surface tension or a function of the latter."

In the following year Morgan[3] published a paper on "Self-Fertilization Induced by Artificial Means," in which he gives an account of some experiments performed on the eggs and sperm of several tunicates.

Under normal conditions the eggs of one individual cannot be fertilized by its own sperm, but, by treating the eggs and sperm with certain amounts of ether, he was able to get large percentages of self-fertilizations.

[1] Loeb, University of California Publications, Physiology, Vol. I., No. 6, pp. 39–53.

[2] Loeb, University of California Publications, Physiology, Vol. I., No. 11, pp. 83–85.

[3] Morgan, *Journal of Experimental Zoölogy*, Vol. I., No. 11, 1904.

Among the possible explanations of this phenomenon Morgan suggests "that the surface tension of the egg is of such a sort that it excludes the sperm of the same individual, but this idea does not appear to give a satisfactory solution, for, aside from the fact that it is difficult to imagine how such a relation could exist, there would also occur cases in which the surface tension of the eggs of other individuals would exclude certain sperm, and this would not appear to be the case. It is true that the addition of the ether to the water may cause a difference in the surface tension of the egg, and it might be made to appear that this was the way in which the self-fertilization is effected in ether solutions, but I cannot believe that this is the explanation, because other experiments show that a considerable amount of ether is necessary to cause self-fertilization."

In a later paper,[1] dealing with the same tunicates, Morgan corrects his previous observations to some extent and shows by a series of experiments that "it is not true that the sperm of a given individual will fertilize equally well the eggs of all other individuals."

As a matter of fact his tables show that the sperm of some individuals is wholly ineffective on the eggs of other individuals, and that all degrees of effectiveness are found. Thus his later experiments serve to offset his chief objection (quoted above) to the idea that surface tension might be an effective factor in controlling the entrance of the sperm into the egg.

Morgan showed that the addition of certain amounts of ether to sea water made the sperm more active, and he inferred from this fact that the activity of the sperm was the factor governing fertilization. On the other hand, he states that eggs have been fertilized by very inactive sperm. This seems to indicate that the activity of the sperm is a subordinate factor in fertilization. Moreover, no matter how slow-moving the sperm might be, some would undoubtedly be able to reach some eggs. The failure to fertilize would not then be due, I believe, to a failure of the sperm to reach the egg-membrane, but rather to a failure to fuse with and penetrate the latter. The fusion, as both Loeb and Morgan have suggested, may very readily be dependent upon surface tension.

[1] Morgan, Biological Bulletin, Vol. VIII., No. 6, 1905.

At the suggestion of Dr. A. P. Mathews I took up this problem to see whether the surface tension, or the condition of the surface of the egg was of importance in determining fertilization. The working hypothesis was that the surface of the egg possessed a certain definite tension that rendered it permeable to sperm of equally definite surface tension. If the tension of either the egg or sperm is altered their fusion might no longer be possible, although the sperm of other species that might chance to possess a more appropriate surface tension might enter.

If the egg be a colloidal system with a membrane of a certain definite surface tension it should be possible so to alter this tension by the use of certain salts, that the sperm of the same species can no longer enter, and after so doing, to reverse the process and restore the original surface tension and the consequent permeability.

According to Mathews[1] the surface tension of colloidal particles or particles in suspension may be increased or decreased by means of salts acting through their tension coefficients. The egg may be regarded as a particle in suspension of which the membrane is electro-negative. It should be possible to increase its surface tension by neutralizing these charges either wholly or in part. A list of the tension coefficients of salts is given in Mathews' paper on the precipitation of colloids and at his suggestion I tried the alkaline earth chlorides, and particularly calcium chloride, as a salt which should increase surface tension, and sodium sulphate as a salt which should have an opposite effect on the surface of the egg.

The eggs of the minnow, *Fundulus heteroclitus*, were chosen for the experiment. These eggs are almost immune to changes in the osmotic pressure of the medium. It was noticed at the outset that eggs of *F. heteroclitus* became incapable of fertilization by their own sperm after exposure to sea water for periods in excess of an hour or in some cases an hour and a quarter. It seemed possible that the cause of this loss of susceptibility to fertilization might be due to certain salts in the sea water. This matter is partially cleared up by what follows.

The first experiments were undertaken to determine which

[1] Mathews, *Ameri an Journal of Physiology* XIV., 1905, p. 203.

salts were most powerful in shortening the period of susceptibility of the egg to its own sperm.

Experiment I. — Large numbers of eggs from various females were stripped into distilled water, mixed thoroughly and transferred to dishes containing respectively 100 c.c. of

A. $\frac{5}{10}$ *n.* $CaCl_2$, *C.* $\frac{6}{10}$ *n.* NaCl,
B. $\frac{5}{10}$ *n.* $MgCl_2$, *D.* $\frac{6}{10}$ *n.* KCl.

These solutions were made approximately isotonic with sea water so as to eliminate the factor of osmosis, although, as stated, these eggs are practically immune to osmotic changes. After periods of five, ten, twenty and thirty minutes' exposure to these solutions, successive lots of eggs — averaging about fifty in number — were taken out, washed in sea water and an attempt made to fertilize them in sea water.

The results of this experiment follow:

A. ($\frac{5}{10}$ *n.* $CaCl_2$). No eggs fertilized at all and all showed coagulation of the blastodisc or plasmolysis of the entire protoplasmic mass, after an exposure of five minutes or longer.

B. ($\frac{5}{10}$ *n.* $MgCl_2$). No eggs fertilized and all showed a vacuolated condition of the protoplasm and a considerable amount of plasmolyses after exposures of five minutes or longer.

C. ($\frac{6}{10}$ *n.* NaCl). About 30 per cent. of all eggs fertilized and cleaved normally and nearly all of these formed embryos, even after the maximum exposure of thirty minutes.

D. ($\frac{6}{10}$ *n.* KCl). From 60 to 75 per cent. of eggs fertilized and cleaved normally, producing normal embryos, even after the maximum exposure of thirty minutes.

From this experiment it will readily be seen that calcium salts are the most active in preventing fertilization, while sodium, and especially potassium salts, have relatively little inhibiting effect.

Experiment II. — In order to test the relative toxicity of these solutions before and after fertilization the following simple experiment was undertaken: A large number of eggs were fertilized in sea water, washed rapidly in distilled water and transferred to the same solution used in Experiment I. After standing in these solutions for thirty minutes they were returned to sea water

and allowed to develop. In every case nearly all eggs developed normally and formed embryos.[1]

It is worthy of note, then, that the egg becomes immensely more resistant to the toxic action of these salts after fertilization.

Several repetitions of Experiment I. showed an invariable order of activity from the most active to the least active —$CaCl_2$, $MgCl_2$, NaCl, KCl. It seemed advisable to include several other chlorides of these groups to see if their activity in inhibiting fertilization would fall in line with the theory of solution tension.

Experiment III. — A large number of eggs were stripped into distilled water, stirred and distributed into seven dishes containing :

A. $\frac{6}{10}$ *n.* LiCl,
B. $\frac{6}{10}$ *n.* NaCl,
C. $\frac{6}{10}$ *n.* KCl,
D. $\frac{5}{10}$ *n.* $MgCl_2$,
E. $\frac{5}{10}$ *n.* $CaCl_2$,
F. $\frac{5}{10}$ *n.* $SrCl_2$,
G. $\frac{5}{10}$ *n.* $BaCl_2$.

All eggs were allowed to remain in these solutions for five minutes, then washed and an attempt made to fertilize them in sea water, abundance of sperm being added in each case. It was found that so few of the eggs cleaved that a percentage basis could not be employed for all of the salts, yet it was quite evident that LiCl had an action between NaCl and $MgCl_2$, while $SrCl_2$ and $BaCl_2$ fell between $MgCl_2$ and $CaCl_2$.

Evidently the concentration of the seven salts used in this experiment was too great so it seemed advisable to determine the minimum concentration and the minimum time of exposure required by the most active of these salts — $CaCl_2$— for complete inhibition of fertilization. The following experiment enabled me to determine this.

Experiment IV.—Solutions of $\frac{1}{10}$ *n.*, $\frac{2}{10}$ *n.*, $\frac{3}{10}$ *n.* and $\frac{4}{10}$ *n.* solutions were made up and lots of eggs were exposed in each concentration for five, ten, twenty and thirty minutes before fertilization was tried. It was found that a $\frac{2}{10}$ *n.* $CaCl_2$ solution, after acting for five minutes allowed only 0 to 4 per cent. of eggs to become fertilized. This concentration and time of exposure should give a good percentage basis for estimating the activity of the seven salts used with reference to $CaCl_2$.

[1] See Mathews, *American Journal of Physiology*, X., 1904, p. 241.

Experiment V. — The same seven salts used in Experiment III. were used in $\frac{2}{10}$ *n.* solutions. The same method of procedure was used throughout as in Experiment III. and the results are given in the following table. The experiment was performed twice and the figures are given side by side for the sake of comparison.

	No. 1.				No. 2.		
	Salt.	No. of Eggs Used.	No. of Cleavages.	Per Cent. of Cleavages.	No. of Eggs Used.	No. of Cleavages.	Per Cent. of Cleavages.
A.	LiCl	72	41	57	38	25	68
B.	NaCl	80	64	80	34	26	76
C.	KCl	92	80	89	45	39	87
D.	$MgCl_2$	78	14	18	50	9	18
E.	$CaCl_2$	108	4	3.7	38	2	5.2
F.	$SrCl_2$	88	15	17	42	6	14.3
G.	$BaCl_2$	82	9	10.9	43	4	9.3

Averaging the percentages of cleavages in the two experiments we find that the salts arrange themselves as follows:—$CaCl_2$, 4.4; $BaCl_2$, 10.1; $SrCl_2$, 15.6; $MgCl_2$, 18; LiCl, 62.5; NaCl, 78; KCl, 88. This corresponds exactly with the order of the precipitating power of these salts on colloidal egg albumin and possibly with the order of the solution tensions of these cations.

These results seem to indicate that the cause of the inhibition of fertilization is of the nature of a precipitation of the colloids of the egg membrane. In order to demonstrate the possibility of rendering the egg impermeable to sperm of the same species and then reversing the coagulation so as to restore its permeability, a $\frac{2}{10}$ *n.* $CaCl_2$ solution was used as a precipitating or coagulating agent and a *m*/10 Na_2So_4 solution was used as a salt which theoretically should reverse the coagulation.

Experiment VI.—A lot of eggs were stripped into 100 c.c. of $\frac{2}{10}$ *n.* $CaCl_2$ solution and after a five minute exposure were divided into two dishes, one containing *m*/10 Na_2So_4 and the other containing sea water as a control. The control eggs were immediately mixed with an abundance of sperm, which was allowed to remain in the dish, but not a single egg cleaved. The eggs transferred to the *m*/10 Na_2So_4 solution were allowed to remain there for twenty minutes and were then transferred to sea water and fertilized. It was found in the course of about three hours that 27 out of 88 eggs had cleaved. The experiment was re-

peated and in this case the control showed two cleavages in 83 eggs while those treated with Na_2So_4 showed twenty normal embryos in 95 eggs.

Evidently, then, the antifertilization effect of the calcium ions on the egg may be reversed by sodium sulphate. Probably the $CaCl_2$ produces the gel condition in the egg membrane and the Na_2SO_4 redissolves the coagulum.

In order to test the hypotheses that the calcium or magnesium ions in sea water have the effect of slowly coagulating the membrane of the egg and of thus rendering it impermeable to its own sperm after a period of an hour or slightly more, the following experiment was undertaken :

Experiment VII. — A lot of eggs were stripped into distilled water, mixed thoroughly and then divided into five dishes containing respectively

A. Sea water.
B. $\frac{6}{10}$ *n*. KCl.
C. $\frac{6}{10}$ *n*. NaCl.
D. Equal parts of *B* and *C*.
E. $\frac{M}{10}$ Na_2SO_4.

After standing one and one-half hours a portion of the eggs from each dish was transferred to sea water, washed and an attempt made to fertilize, with the following result :

	Solution Used.	No. of Eggs Used.	No. of Embryos Formed.	Per Cent. of Embryos.
A.	Sea water.	78	0	0
B.	$\frac{6}{10}$ *n*. KCl.	45	14	31.1
C.	$\frac{6}{10}$ *n*. NaCl.	52	3	5.7
D.	Equal parts of *A* and *B*.	58	9	15.5
E.	M/10 Na_2SO_4.	63	42	66.6

After standing two and one-half hours another portion of the eggs was transferred from the original five dishes, washed and fertilized in sea water with the following result :

	Solution Used.	No. of Eggs Used.	No. of Embryos Formed.	Per Cent. of Embryos.
A.	Sea water.	68	0	0
B.	$\frac{6}{10}$ *n*. KCl.	51	8	15.7
C.	$\frac{6}{10}$ *n*. NaCl.	41	2	4.8
D.	Equal parts of *A* and *B*.	43	2	4.6
E.	M/10 Na_2SO_4.	58	14	24.1

After standing three and one-half hours the remainder of the eggs were transferred to sea water and fertilized, but in no case were any embryos formed.

The above series of experiments goes to show that the term of permeability of the egg membrane may be considerably prolonged by the exclusion of the calcium and magnesium salts. In no case, however, were the eggs capable of fertilization after standing for three and one half hours. It seems quite probable that the membrane is affected by internal changes in the protoplasm, which must be very active at this period. It is also to be noted that the $\frac{M}{10}Na_2SO_4$ solution helps the egg to retain its permeability longer than any of the ingredients of sea water. Further experimentation might lead to the discovery of a medium that would very greatly prolong the period of susceptibility to fertilization.

Conclusion. — These experiments show clearly enough that the membrane of the egg of *Fundulus heteroclitus* is subject to manipulation by the use of cations and anions, so that it becomes permeable or impermeable to its own sperm at the will of the investigator. I am unable to formulate any explanation of these changes in the condition of the membrane except that of a reversible coagulation and the consequent reversible surface tension.

When the surface tension of the egg is increased by coagulation of its colloids the sperm probably stands out on the surface of this altered membrane much as a minute droplet of water would stand out on the surface of an oil globule. Under ordinary conditions, however, the sperm surface and the egg surface are so adjusted to one another that they fuse on contact, and the sperm substance is engulfed by that of the egg.

We may suppose, then, that each species has a surface tension for the egg that is exactly suited to that of its own sperm and that, if altered, it becomes no longer suited to its own sperm but may become suited to other closely related sperm.

Loeb's results, cited at the beginning of this paper, offer evidence in favor of this view.

In the case of hermaphroditic animals, such as the tunicates with which Morgan worked, the condition is undoubtedly differ-

ent. It seems probable that the egg and sperm of the same individual have a slightly incompatible surface tension of egg and sperm, an incompatibility that may be removed by the action of ether. It may also be readily understood why the sperm of one individual is not equally effective on the eggs of all other individuals, for some eggs have a surface tension more suitable to that of the sperm than others.

If in any way one could overcome the difficulties of surface tension and could inject the sperm of unrelated forms beyond the egg membrane without injuring the egg, we might get some very significant results. This is a field as yet unexplored but I hope not unpromising. These experiments will probably be continued with other eggs and over a more extended field.

Summary.

1. The calcium salts are the most active of the salts found in sea water in inhibiting fertilization. They are also the most active salts in precipitating colloids. Hence there is probably a causal relation between the precipitation of colloids and the inhibition of fertilization.

2. The seven salts $CaCl_2$, $BaCl_2$, $SrCl_2$, $MgCl_2$, LiCl, NaCl, and KCl inhibit fertilization in the order of their tension coefficients. Thus the egg is acted upon as if it were a colloidal system.

3. Supposing the egg to be a colloidal system with a membrane negatively charged it should be possible to precipitate or coagulate this membrane by salts of high positive tension coefficients, such as $CaCl_2$, and to redissolve this precipitate or coagulum by the use of salts of high negative tension coefficients, such as Na_2So_4. This was done successfully, fertilization being inhibited by $CaCl_2$ and restored by Na_2SO_4.

4. This alteration in the character of the membrane must involve an alteration in the surface tension that controls the entrance of the sperm.

5. The short term of susceptibility of the egg to fertilization by its own sperm, when the eggs are allowed to stand in sea water is probably due to the presence of calcium salts that slowly coagulate the membrane and thus increase its surface tension. The period of susceptibility to fertilization is considerably pro-

longed in the absence of calcium salts and especially in a medium of Na_2So_4.

6. These experiments and those of Loeb and Morgan seem to indicate that the relative surface tension of the egg and of the sperm is one important factor governing fertilization.

MARINE BIOLOGICAL LABORATORY,
WOOD'S HOLL, MASS., August, 1905.